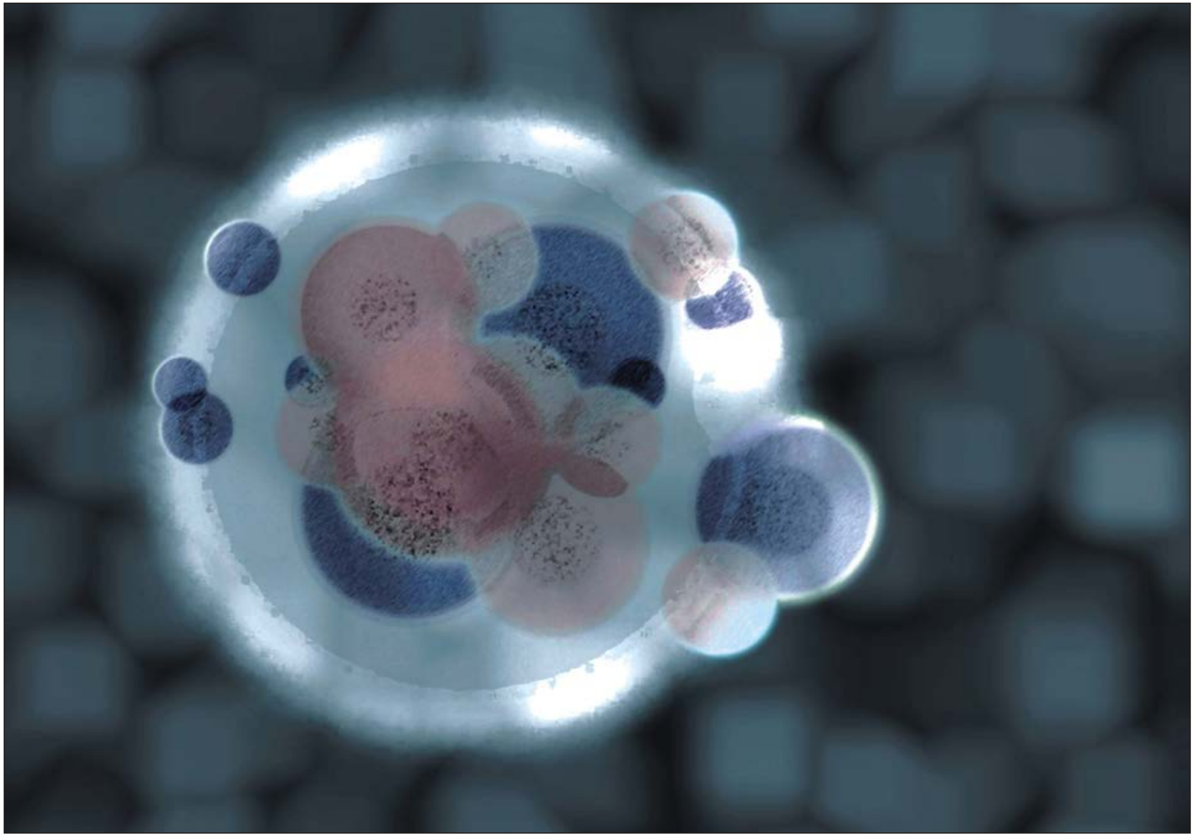

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Molecular Detection of Three Viral Diseases in Honeybees (*Apis mellifera* L.) Colonies Infested with Mite (*Varroa destructor*) in Jordan

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Abstract: Little information exists in Jordan about the presence of viral diseases in honey bee (*Apis mellifera*) colonies and their association with *Varroa destructor* mites. The first investigation using molecular techniques of honey bees colonies infested with *Varroa* mites for the presence of three viruses in Jordan is reported. Samples of adult bees, larvae and *Varroa* mites were collected from twenty four colonies represented seven different locations of Jordan. Infestation percentages were 54.2% for Israeli Acute Paralysis Virus (IAPV) followed by 11.1% for Deformed Wing Virus (DWV) and 4.2% for Black Queen Cell Virus (BQCV). IAPV and DWV were detected in adult bees, larvae and *Varroa*, while, BQCV was found only in adult bees and *Varroa*. The results were confirmed by analyzing the samples with reverse transcription-PCR (RT-PCR) for the three viruses surveyed using specific primers. This is the first molecular detection of these three viruses infecting honey bees in Jordan.

Key words: *Apis mellifera*, honeybee viruses, RT-PCR, *Varroa destructor*

INTRODUCTION

Honeybee, *Apis mellifera* L., is one of the most important economical insects by both its various products and by contribution to the pollination of agricultural plants. The beekeeping industry plays a key role in agricultural production in Jordan, the total production value of 12 bee-pollinated crops was 117.4 million dollars in 2005 of which, 50.7 million dollars could be directly attributed to pollination by honeybees (Haddad *et al*., 2008). However, the health and vigor of honeybee colonies are threatened by numerous parasites and pathogens, including viruses, bacteria, protozoa and mites.

Among pathogens attacking honeybees, viruses are probably the least understood because of the lack of information about the dynamics underlying viral disease outbreaks (Chen *et al*., 2006). Honey bee was subjected to many viral infections and a total of 18 viruses have been identified and characterized in this species (Shen *et al*., 2005). The occurrence of Israeli Acute Bee Paralysis Virus (IAPV), Kashmir Bee Virus (KBV), Chronic Bee Paralysis Virus (CBPV) and Acute Bee Paralysis Virus (ABPV) have been proved in Jordan by Al-Abbadi *et al*. (2010a, b). The ABPV, Sac Brood Virus (SBV) and Deformed Wing Virus (DWV) were detected only in Ajloun area by Haddad *et al*. (2008). The existing of SBV, Black Queen Cell Virus (BQCV) and the differences in the distribution pattern of these two viruses in different regions of Jordan had determined (Al-Abbadi *et al*., 2013).

The ectoparasitic mite *Varroa destructor* is an obligate parasite and considered a major pest of the honeybee *A. mellifera*. Colonies infested with *V. destructor* develop the parasitic mite syndrome and ultimately collapse if left untreated (Shimanuki *et al*., 1994). *V. destructor* has been confirmed as a vector in transmitting and activating bee virus infections and it is well established that viruses vectored by *V. destructor* play an important role in *Varroa*- induced colony collapse (Martin, 2001; Shen *et al*., 2005). All reproduction of *Varroa* mites occurs in the brood cells and only the adult females survive after the bee emerges. *Varroa* mites suck the hemolymph from adults and developing pupae of honeybees, thereby weakening the bees and shortening their life span (Shen *et al*., 2005). Because the parasitic mite *V. destructor* feeds and moves regularly between brood and adult bees, these mites have the potential to act as either biological or mechanical vectors of bee viruses. While bee viruses were noticed prior to the arrival of *V. destructor* in *A. mellifera* populations (Bailey, 1976), viral disease outbreaks have often been reported to be associated with mite infestations.

A most crucial stage in the dynamics of virus infections is the mode of virus transmission. In general,

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transmission of viruses can occur through two pathways: vertical and horizontal transmission (Berenyi *et al*., 2007; Forgach *et al*., 2008). In vertical transmission, viruses are transmitted from adults to their offspring, while horizontal transmission occurs among individuals of the same generation. Horizontal transmission is also very likely among adult bees and from adult workers to larvae through contaminated food resources, because both viruses have been detected in all developmental stages and food sources (brood food, honey, pollen and royal jelly). Furthermore, it was demonstrated that mites were another possible route of horizontal transmission, as both viruses were detected in mites and their saliva (Sumpter and Martin, 2004).

To increase understanding of the relationship among viruses, mites and colony decline and the tripartite relationships among bees, two viruses (KBV and SBV) and *Varroa* mites have been investigated systematically by Shen *et al*. (2005). By using Enzyme-linked Immunosorbent Assay (ELISA) and Reverse Transcription-Polymerase Chain Reaction (RT-PCR), the presence of KBV and SBV was studied comparatively in different developmental stages and castes of bees. The results demonstrated that KBV may persist as a viral genome with extremely low levels of viral-capsid proteins and that KBV and SBV can co-infect honeybees. Moreover, the study indicated the presence of KBV and SBV RNAs in both queens and eggs by RT-PCR, suggesting a route of transovarial transmission. By using RT-PCR, several honeybee viruses were detected in mite samples collected from honeybee colonies from different countries (Hung *et al*., 2000; Chen *et al*., 2004; Yue and Genersch, 2005; Chantawannakul *et al*., 2006; Gulmez *et al*., 2009; Gisder *et al*., 2009).

Although, honeybee viruses are reported from many countries (Allen and Ball, 1996; Chen *et al*., 2005; Lanzi *et al*., 2006; Teixeira *et al*., 2008; Berenyi *et al*., 2006) and much research has been done on the characterization of bee viruses, little is known about the transmission routes of bee viruses and the relationship with *Varroa* mites. Six honeybee viruses were detected in Jordan (Al-Abbadi *et al*., 2010a, b; 2013; Haddad *et al*., 2008), whereas there are no reports about causative agents of the viral honeybee diseases in Jordan.

In this study, RT-PCR was used to detect the association between honeybee viruses (Israeli bee paralysis virus, black queen cell virus, deformed wing virus) and *Varroa* mite collected from Jordanian honeybee colonies.

MATERIALS AND METHODS

Seventy two samples were collected from 24 colonies of different apiaries that located in seven different regions of Jordan including Yadoda, Baqa, Alsalt, Abu-Nuseer, Shuna Janobya, Wadi Shueeb and Humrat Alsahen.

The collected samples represented honeybee colonies infected with *Varroa* mites with or without apparent symptoms of virus infection. The samples of each colony included; honeybee adults, larvae and *Varroa* mites. Each sample was consisted of 15-20 honeybee adults and larvae and about 20 *Varroa* mites. The samples were kept in separate test tubes and stored at -80°C until investigation. The frozen samples were homogenized in liquid nitrogen and the RNA was then extracted by employing QIAamp® viral RNA Kit (QIAGEN) according to the manufactures instructions. The extracted RNA was used for the RT-PCR testes.

Amplification was carried out in a total volume of 25 μL, where 2.4 μL of template RNA (diluted 2:20 μL) was added to the master mix that contained: 5 units (0.1 μL) of reverse transcriptase, 1 μL of dNTPs, 0.5 μL $MgSO_4$, 2 μL of 5X RT-PCR and 3 μL of 10X Taq-PCR buffer, 5 units (0.1 μL) of Taq-polymerase, 1.0 μL of 10 μM of each forward and reverse primer. Doubled distilled water was added to the final volume.

The IAPV primer pair was IAPVF (5'-AGACACCAATCACGGACCTCAC-3') and IAPVR (5'-AGATTTGTCTGTCTCCCAGTGCACAT-3') to amplify 475 bp segment. The BQCV primer pair was BQCVF (5-'GGAGATGTATGCGCTTTATCGAG-3') and BQCVR (5' CACCAACCGCATAATAGCGATTG3') to amplify 316 bp segment. The DWV primer pair was DWVF (5-' CTCGTCATTTTGTCCCGACT-3') and DWVR (5'TGCAAAGATGCTGTCAAACC- 3') to amplify 424 bp segment (Table 1).

Reverse transcription and amplification were conducted with a continuous RT-PCR method using the

Table 1: Primers used for the detection of three honeybee viruses

Virus name	Primer sequence (5´-3´)	Amplicon size (bp)	References
Israeli acute paralysis virus (IAPV)			
F	AGACACCAATCACGGACCTCAC	475	Maori *et al*. (2007)
R	AGATTTGTCTGTCTCCCAGTGCACAT		
Black queen cell virus (BQCV)			
F	GGAGATGTATGCGCTTTATCGAGI	316	Topley *et al*. (2005)
R	CACCAACCGCATAATAGCGATTGI		
Deformed wing virus (DWV)			
F	CTCGTCATTTTGTCCCGACT	424	Williams *et al*. (2009)
R	TGCAAAGATGCTGTCAAACC		

following program: RT for 45 min at 46°C followed by 1 cycle of 2 min at 94°C, followed by 40 cycles of 30 sec at 94°C, 55 sec at (56.5°C for IAPV, 54.5°C for BQCV and 54°C for DWV) and 1 min at 72°C, followed by 10 min at 72°C for final extension. The reaction samples were detected by 1% agarose gel electrophoresis using 10 µL of the PCR products, staining by ethidium bromide and photographing under UV light.

RESULTS AND DISCUSSION

Varroa destructor infestation of honeybees was noticed in Jordan in 1986 when the beekeeping sector faced major losses and as a result over 50% of the beehives dyed. This happened one year after the first notification of the ectoparasitic *Varroa* mite in honeybee colonies (Nazer and Rateb, 1991). However, there is no report for the presence of any virus infection reported to date. In this study, 24 apiaries with total of 72 samples were collected from seven different locations of Jordan (Yadoda, Baqa, Alsalt, Abu-Nuseer, Shuna Janobya, Wadi Shueeb and Humrat Alsahen). Thirty nine samples were detected positive for IAPV infection (13 adults, 13 larvae and 13 *Varroa*) that represented all the seven locations. BQCV detected in larvae and varroa within two locations (Shuna Janobya and Wadi Shueeb). DWV detected in eight samples (1 adult, 4 larvae, 3 varroa) within four locations (Baqa, Alsalt, Abu-Nuseer and Wadi Shueeb) (Table 2).

The PCR primer for IAPV generated an amplicon of about 475 bp (Fig. 1) in 54.2% samples (39 out of 72) from the mite-infested colony samples, which is about the same size as the IAPV amplicon reported by Maori *et al*. (2007). The BQCV primer generated an amplified segment of about 316 bp in 4.2% (3 out of 72 samples), this size mentioned by Topley *et al*. (2005) (Fig. 2). The PCR amplification product with DWV primer was about 424 bp in 11.1% (8 out of 72 samples), similar amplicon size was reported by Williams *et al.* (2009) (Fig. 3). Such infections with IAPV add to the pathology of *V. destructor* could play a major role in honeybee colonies collapse in Jordan. The results also demonstrated that IAPV is able to replicate in adults, larvae and varroa and so as for DWV and this is in agreement with the findings of Guzman-Novoa *et al.* (2012) and Di Prisco *et al*. (2011). The BQCV was detected in adults and varroa only. These findings supported the horizontal transmission of the viruses within honeybee colonies. Also, these viruses could usually cause unapparent infections and may not be perceived by beekeepers for many years.

BQCV was originally found in dead honeybee queen larvae and pupae (Bailey and Woods, 1977). This support the results that indicated the presence of only one infected adult bee and many others varroa. This virus was very prevalent, especially in adult bees, whereas infections in pupae were scarcely detected (Tentcheva *et al*., 2004).

Table 2: Samples numbers, locations and RT-PCR detection of the three viruses within each honeybee and *Varroa* samples

		IAPV			BQCV			DWV		
Colony No.	Location	A	L	V	A	L	V	A	L	V
1	Yadoda	+	+	+						
2	Yadoda									
3	Yadoda	+								
4	Yadoda	+								
5	Yadoda									
6	Baqa									
7	Baqa		+	+						
8	Baqa	+	+	+						
9	Baqa	+	+	+						
10	Baqa	+	+	+					+	
11	Alsalt	+	+							+
12	Alsalt	+	+	+					+	+
13	Alsalt			+						
14	Alsalt								+	
15	Alsalt	+		+						
16	Abu-Nuseer	+	+	+						
17	Abu-Nuseer	+							+	
18	Abu-Nuseer	+	+							+
19	Shuna Janobya						+			
20	Shuna Janobya		+	+	+		+			
21	Wadi Shueeb	+	+	+				+		
22	Humrat Alsahen		+							
23	Humrat Alsahen		+	+						
24	Baqa			+						

A: Adult, L: Larvae, V: *Varroa*

Fig. 1: Agarose gel electrophoresis for detection of IAPV by RT-PCR in six honeybee samples collected from different regions. Lane M: 100 bp marker, lanes represented samples (1-6) of adults (A), larvae (L) and *Varroa* (V)

Fig. 2: Agarose gel electrophoresis for detection of BQV by RT-PCR in six honeybee samples collected from different regions. Lane M: 100 bp marker, lanes represented samples (19-24) of adults (A), larvae (L) and *Varroa* (V)

Fig. 3: Agarose gel electrophoresis for detection of DWV by RT-PCR in six honeybee samples collected from different regions. Lane M: 100 bp marker, lanes represented samples (13-18) of adults (A), larvae (L) and *Varroa* (V)

These results indicated that if any of the IABPV, DWV and BQCV is present in honey bee colony or in the apiary, they could be transmitted by both honeybees and/or *Varroa* mites. It may also be concluded that the presence of *Varroa* mite in any infected colony in the apiary may play an important role in the transmission of the viruses in all apiaries through the foraging behavior of honeybees. Beekeepers should take care when transmitted bee brood, pollen grains and honeybee combs from one colony to another to prevent the infection of *Varroa* or viral diseases.

In conclusion, this study demonstrated that infected mites play an important role in transmission of viruses and those *Varroa* mites are capable of transmitting IAPV, BQCV and DWV among honeybees. The present study proves that different honeybee viruses are circulating in Jordan apiaries; the detection of IAPV, BQCV and DWV in different locations and colonies is valuable as it helps us to choose which virus to focus on especially the IAPV. This investigation also clarifies the power of molecular techniques in viral studies, the ability of RT-PCR to detect viruses in individual adult bees, larvae and mites should prove of great use in future studies directed at the epidemiology of many other honey bee viruses.

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REFERENCES

Al-Abbadi, A.A., D.S. Hassawi, S.A. Abu-Mallouh and M.S. Al-Mazra'awi, 2010a. Novel detection of Israel acute paralysis virus and Kashmir bee virus from honeybees *Apis mellifera* L. (Hymenoptera: Apidae) of Jordan using reverse transcriptase PCR technique. Applied Entomol. Zool., 45: 183-190.

Al-Abbadi, A.A., D.S. Hassawi, S. Abu-Romman and S.A. Abu-Mallouh, 2010b. Detection of chronic and acute bee paralysis viruses from jordanian honeybee apiaries by reverse transcriptase PCR. J. Food Agric. Environ., 8: 1016-1019.

Al-Abbadi, A.A., D.S. Hassawi, S.A. Abu-Mallouh and M. Obeidat, 2013. Detection of sac brood and black queen cell viruses from honeybee colonies of Jordan using RT-PCR technique. Jordan J. Agric. Sci., 9: 201-210.

Allen, M. and B. Ball, 1996. The incidence and world distribution of honey bee viruses. Bee World, 77: 141-162.

Bailey, L., 1976. Viruses Attacking the Honeybee. In: Advances in Virus Research, Lauffer, M.A., F.B. Bang, K. Maramorosch and K.M. Smith (Eds.). Vol. 20, Academic Press, New York, USA., pp: 271-304.

Bailey, L. and R.D. Woods, 1977. Two more small RNA viruses from honey bees and further observations on sacbrood and acute bee-paralysis viruses. J. Gen. Virol., 25: 175-186.

Berenyi, O., T. Bakonyi, I. Derakhshifar, H. Koglberger and H. Nowotny, 2006. Occurrence of six honeybee viruses in diseased Austrian apiaries. Applied Environ. Microbiol., 72: 2414-2420.

Berenyi, O., T. Bakonyi, I. Derakhshifar, H. Koglberger and G. Topolska *et al*, 2007. Phylogenetic analysis of deformed wing virus genotypes from diverse geographic origins indicates recent global distribution of the virus. Applied Environ. Microbiol., 73: 3605-3611.

Chantawannakul, P., L. Ward, N. Boonham and M. Brown, 2006. A scientific note on the detection of honeybee viruses using real-time PCR (TaqMan) in Varroa mites collected from a Thai honeybee (*Apis mellifera*) apiary. J. Invertebrate Pathol., 91: 69-73.

Chen, Y., J.S. Pettis, J.D. Evans, M. Kramer and M.F. Feldlaufer, 2004. Transmission of Kashmir bee virus by the ectoparasitic mite *Varroa destructor*. Apidologie, 35: 441-448.

Chen, Y.P., J.A. Higgins and M.F. Feldlaufer, 2005. Quantitative real-time reverse transcription-PCR analysis of deformed wing virus infection in the honeybee (*Apis mellifera* L.). Applied Environ. Microbiol., 71: 436-441.

Chen, Y.P., J.S. Pettis, A. Collins and M.F. Feldlaufer, 2006. Prevalence and transmission of honeybee viruses. Applied Environ. Microbiol., 72: 606-611.

Di Prisco, G., F. Pennacchio, E. Caprio, H.F. Boncristiani Jr., J.D. Evans and Y. Chen, 2011. Varroa destructor is an effective vector of Israeli acute paralysis virus in the honeybee, *Apis mellifera*. J. Gen. Virol., 92: 151-155.

Forgach, P., T. Bakonyi, Z. Tapaszti, N. Nowotny and M. Rusvai, 2008. Prevalence of pathogenic bee viruses in Hungarian apiaries: Situation before joining the European Union. J. Invertebrate Pathol., 98: 235-238.

Gisder, S., P. Aumeier and E. Genersch, 2009. Deformed wing virus: Replication and viral load in mites (*Varroa destructor*). J. Gen. Virol., 90: 463-467.

Gulmez, Y., A. Bursali and S. Tekin, 2009. First molecular detection and characterization of deformed wing virus (DWV) in honeybees (*Apis mellifera* L.) and mite (*Varroa destructor*) in Turkey. Afr. J. Biotechnol., 8: 3689-3702.

Guzman-Novoa, E., M.M. Hamiduzzaman, L.G. Espinosa-Montano, A. Correa-Benitez, R. Anguiano-Baez and R. Ponce-Vazquez, 2012. First detection of four viruses in honey bee (*Apis mellifera*) workers with and without deformed wings and *Varroa destructor* in Mexico. J. Apicult. Res., 51: 342-346.

Haddad, N., M. Brake, H. Migdadi and J.R. de Miranda, 2008. First detection of honey bee viruses in Jordan by RT-PCR. Jordan J. Agric. Sci., 4: 242-247.

Hung, A.C.F., C.Y.S. Peng and H. Shimanuki, 2000. Nucleotide sequence variations in Kashmir bee virus isolated from *Apis mellifera* L. and *Varroa jacobsoni* oud. Apidologie, 31: 17-23.

Lanzi, G., J.R. de Miranda, M.B. Boniotti, C.E. Cameron and A. Lavazza *et al*., 2006. Molecular and biological characterization of deformed wing virus of honeybees (*Apis mellifera* L.). J. Virol., 80: 4998-5009.

Maori, E., E. Tanne and I. Sela, 2007. Reciprocal sequence exchange between non-retro viruses and hosts leading to the appearance of new host phenotypes. Virology, 362: 342-349.

Martin, S.J., 2001. The role of *Varroa* and viral pathogens in the collapse of honeybee colonies: A modelling approach. J. Applied Ecol., 38: 1082-1093.

Nazer, I.K. and J.M. Rateb, 1990. Status of honey bee diseases and pests in Jordan. Adv. Environ. Biol., 1: 1-3.

Shen, M., L. Cui, N. Ostiguy and D. Cox-Foster, 2005. Intricate transmission routes and interactions between picorna-like viruses (Kashmir bee virus and sacbrood virus) with the honeybee host and the parasitic *Varroa* mite. J. Gen. Virol., 86: 2281-2289.

Shimanuki, H., N.W. Calderone and D.A. Knox, 1994. Parasitic mite syndrome: The symptoms. Am. Bee J., 134: 827-828.

Sumpter, D.J.T. and S.J. Martin, 2004. The dynamics of virus epidemics in Varroa-infested honey bee colonies. J. Anim. Ecol., 73: 51-63.

Teixeira, E.W., Y. Chen, D. Message, J. Pettis and J.D. Evans, 2008. Virus infections in Brazilian honey bees. J. Invertebrate Pathol., 99: 117-119.

Tentcheva, D., L. Gauthier, N. Zappulla, B. Dainat, F. Cousserans, M.E. Colin and M. Bergoin, 2004. Prevalence and seasonal variations of six bee viruses in *Apis mellifera* L. and *Varroa destructor* mite populations in France. Applied Environ. Microbiol., 70: 7185-7191.

Topley, E., S. Davison, N. Leat and M. Benjeddou, 2005. Detection of three honeybee viruses simultaneously by a single multiplex reverse transcriptase PCR. Afr. J. Biotechnol., 4: 763-767.

Williams, G.R., R.E.L. Rogers, A.L. Kalkstein, B.A. Taylor, D. Shutler and N. Ostiguy, 2009. Deformed wing virus in western honey bees (*Apis mellifera*) from Atlantic Canada and the first description of an overtly-infected emerging queen. J. Invertebrate Pathol., 101: 77-79.

Yue, C. and E. Genersch, 2005. RT-PCR analysis of deformed wing virus in honeybees (*Apis mellifera*) and mites (*Varroa destructor*). J. Gen. Virol., 86: 3419-3424.